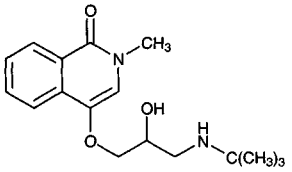
# Tilisolol



**Molecular formula:** $C_{17}H_{24}N_2O_3$
**Molecular weight:** 304.39
**CAS Registry No.:** 85136-71-6
**Merck Index:** 9579

## SAMPLE

**Matrix:** aqueous humor, blood, vitreous body
**Sample preparation:** Aqueous humor, vitreous body. Mix 200 µL aqueous humor or vitreous body with 20 µL 1 M HCl and 300 µL 100 µg/mL o-ethoxybenzamide in MeOH. Centrifuge at 12000 g for 15 min and inject a 50 µL aliquot of the supernatant. Plasma. Mix 700 µL plasma with 300 µL 2 M perchloric acid and centrifuge at 12000 g for 15 min. Shake 800 µL supernatant with 200 µL 5 M NaOH and 6 mL chloroform for 15 min, centrifuge at 650 g for 15 min. Remove a 5 mL aliquot of the organic layer and mix with 100 µL 100 µg/mL o-ethoxybenzamide in MeOH, evaporate under reduced pressure. Dissolve residue in 250 µL MeOH: phosphate-buffered saline 20:80, inject an aliquot.

## HPLC VARIABLES

**Column:** 150 × 4.6 Cosmosil 5 C18-P
**Mobile phase:** MeOH:50 mM $NaH_2PO_4$ 37:63
**Flow rate:** 1
**Injection volume:** 50
**Detector:** F ex 315 em 420

## CHROMATOGRAM

**Internal standard:** o-ethoxybenzamide

## KEY WORDS

rabbit; plasma

## REFERENCE

Sasaki,H.; Ichikawa,M.; Kawakami,S.; Yamamura,K.; Nishida,K.; Nakamura,J. In situ ocular absorption of tilisolol through ocular membranes in albino rabbits, *J.Pharm.Sci.*, **1996**, *85*, 940–943.

## SAMPLE

**Matrix:** blood
**Sample preparation:** 1 mL Plasma + 1 mL 1 M perchloric acid, shake for a few min, centrifuge at 1600 g for 10 min. Remove a 1.5 mL aliquot of the supernatant and add it to 500 µL 6 M NaOH and 5 mL chloroform, vortex for 1 min, centrifuge at 700 g for 5 min. Remove 4 mL of the chloroform extract and add it to 1 mL 400 ng/mL reserpine in chloroform, evaporate to dryness under reduced pressure, reconstitute with 200 µL MeOH, inject a 40 µL aliquot.

## HPLC VARIABLES

**Column:** 400 × 3 5 µm Nucleosil C18
**Mobile phase:** MeCN:MeOH:acetic acid:water 30:10:0.4:15
**Flow rate:** 1.2
**Injection volume:** 40
**Detector:** F ex 313 em 420

## CHROMATOGRAM

**Retention time:** 8.4
**Internal standard:** reserpine (14.7)
**Limit of quantitation:** 2 ng/mL

## KEY WORDS

plasma; dog; human; pharmacokinetics

### REFERENCE

Yonezawa,K.; Sato,K.; Kobayashi,A. High-performance liquid chromatography of a new β-blocker, 4-[3-(tert.-butylamino)-2-hydroxypropoxy]-N-methylisocarbostyril hydrochloride, in plasma using fluorometric detection, *J.Chromatogr.*, **1985**, *339*, 219–222.

### SAMPLE

**Matrix:** perfusate
**Sample preparation:** 50 μL Perfusate + 50 μL pH 7.4 phosphate-buffered saline or 100 mM HCl + 100 μL 300 μg/mL o-ethoxybenzamide in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot of the supernatant.

### HPLC VARIABLES

**Column:** 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
**Mobile phase:** MeOH:50 mM $NaH_2PO_4$ 37:63
**Flow rate:** 1
**Injection volume:** 50
**Detector:** F ex 315 em 420

### CHROMATOGRAM

**Internal standard:** o-ethoxybenzamide

### KEY WORDS

rabbit

### REFERENCE

Sasaki,H.; Igarashi,Y.; Nagano,T.; Nishida,K.; Nakamura,J. Different effects of absorption promoters on corneal and conjunctival penetration of ophthalmic β-blockers, *Pharm.Res.*, **1995**, *12*, 1146–1150.

### SAMPLE

**Matrix:** solutions
**Sample preparation:** 50 μL Solution + 50 μL pH 7.4 PBS + 100 μL 300 μg/mL o-ethoxybenzamide in MeOH, centrifuge at 12000 g for 10 min, inject a 50 μL aliquot.

### HPLC VARIABLES

**Column:** 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
**Mobile phase:** MeOH:50 mM $NaH_2PO_4$ 37:63
**Flow rate:** 1
**Injection volume:** 50
**Detector:** F ex 315 em 420

### CHROMATOGRAM

**Internal standard:** o-ethoxybenzamide

### KEY WORDS

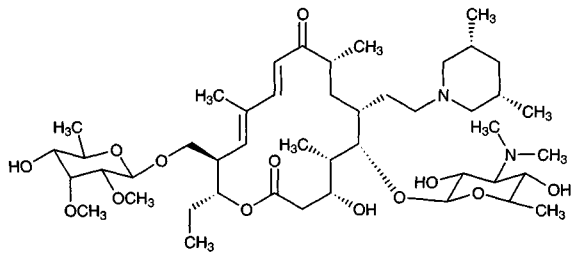
buffer; Earle's balanced salt solution

### REFERENCE

Sasaki,H.; Igarishi,Y.; Nishida,K.; Nakamura,J. Intestinal permeability of ophthalmic β-blockers for predicting ocular permeability, *J.Pharm.Sci.*, **1994**, *83*, 1335–1338.

# Tilmicosin

**Molecular formula:** $C_{46}H_{80}N_2O_{13}$
**Molecular weight:** 869.15
**CAS Registry No.:** 108050-54-0
**Merck Index:** 9580



cis-form

## SAMPLE

**Sample preparation:** Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g $K_2HPO_4$ and 1.046 g $KH_2PO_4$ in 1 L water.)

## HPLC VARIABLES

**Guard column:** 4 × 4 5 μm C18
**Column:** 125 × 4 5 μm Lichrospher RP18
**Mobile phase:** Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
**Flow rate:** 0.5
**Injection volume:** 200
**Detector:** MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 677.4-869.6 in NCI and 869.6-679.4 in PCI

## CHROMATOGRAM

**Retention time:** 5.7
**Limit of detection:** 50 μg/kg

## OTHER SUBSTANCES

**Extracted:** erythromycin, josamycin, spiramycin, tylosin

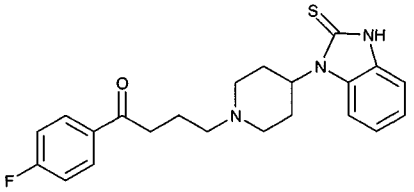
## KEY WORDS

muscle; cow; SPE

## REFERENCE

Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

# Timiperone



**Molecular formula:** $C_{22}H_{24}FN_3OS$
**Molecular weight:** 397.52
**CAS Registry No.:** 57648-21-2
**Merck Index:** 9584

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 500 µL Plasma + 100 µL 250 ng/mL IS in 100 mM pH 3.5 phosphate buffer + 500 µL 500 mM pH 8.5 phosphate buffer, extract with 2.5 mL heptane:isoamyl alcohol 98:2. Shake for 5 min, centrifuge at 1700 g for 10 min. Mix 2.0 mL organic layer with 100 µL 3 M acetic acid, shake for 20 min, centrifuge at 1700 g for 10 min. Aspirate organic layer, inject a 20 µL aliquot of the aqueous layer.

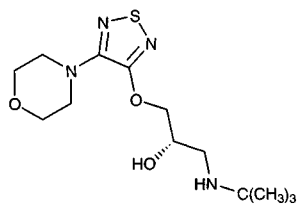
**HPLC VARIABLES**
**Column:** 150 × 4.6 5 µm EicomPak MA-5ODS (Eicom, Japan)
**Mobile phase:** MeCN:MeOH:buffer 20:15:65 containing 500 µg/L (?) disodium EDTA (Buffer was 100 mM $KH_2PO_4$ adjusted to pH 3.5 with 100 mM phosphoric acid.)
**Flow rate:** 1
**Injection volume:** 20
**Detector:** E, Eicom ECD-100, glassy carbon electrode +1 V, Ag/AgCl reference electrode

**CHROMATOGRAM**
**Retention time:** 11.3
**Internal standard:** spiperone (14.9)
**Limit of quantitation:** 500 pg/mL

**KEY WORDS**
plasma; rat

**REFERENCE**
Takayasu,T.; Kakubari,I.; Fukamachi,A.; Mafune,E.; Takasugi,N.; Takayama,K.; Nagai,T. Determination of timiperone in rat plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1996**, *679*, 161–165.

# Timolol



**Molecular formula:** $C_{13}H_{24}N_4O_3S$
**Molecular weight:** 316.42
**CAS Registry No.:** 26839-75-8, 91524-16-2 (hemihydrate), 26921-17-5 maleate
**Merck Index:** 9585
**Lednicer No.:** 2 272

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Condition a 3 mL 500 mg Bakerbond C18 SPE cartridge with 2.5 mL MeOH and 2.5 mL water. Urine. 200 µL Urine + 800 µL 100 mM pH 7.4 phosphate buffer + 100 µL 50 mM HCl + 100 µL 20 ng/mL IS in 50 mM HCl, mix. Add to the SPE cartridge. Wash with 2.5 mL 100 mM pH 10.6 $K_2HPO_4$, then with 2.5 mL water. Elute with 2.5 mL MeOH, evaporate the eluate to dryness at 50°. Reconstitute in 100 µL MeCN:MeOH:0.1% TFA 27:7:66 sonicate for 20 min, inject an aliquot. Plasma. 1 mL Plasma + 100 µL 50 mM HCl + 100 µL 20 ng/mL IS in 50 mM HCl + 2 mL 100 mM pH 10.6 $K_2HPO_4$, mix. Add to the SPE cartridge. Wash with 2.5 mL 100 mM pH 10.6 $K_2HPO_4$ and with 2.5 mL water. Elute with 2.5 mL MeOH,

evaporate the eluate to dryness under reduced pressure at 50°. Add 20 μL 1% pyridine in THF to the residue, vortex, add 40 μL 20% phosgene in toluene, heat at 85° for 2 h. Remove the excess reagent under nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:0.1% TFA in water 36:6:58, sonicate for 20 min. Inject an aliquot. (Phosgene reacts with the hydroxy and secondary amino groups of timolol to form the oxazolidinone derivative.)

**HPLC VARIABLES**
**Guard column:** 20 Supelco Pelliguard LC-CN
**Column:** 50 × 4.6 3 μm Spherisorb CN
**Mobile phase:** MeCN:MeOH:0.1% TFA in water 45:5:50
**Flow rate:** 0.5
**Injection volume:** 10-50
**Detector:** MS, Sciex API IIIplus triple quadrupole, nebulizer 500°, nebulizing gas air 80 psi, curtain gas nitrogen, collision gas argon 50 eV, multiplier 3500 V, positive mode ionization

**CHROMATOGRAM**
**Retention time:** 5.53
**Internal standard:** N-isopropyl timolol (4.88)
**Limit of quantitation:** 200 pg/mL (plasma), 50 ng/mL (urine)

**KEY WORDS**
plasma; urine; dog; SPE; pharmacokinetics; derivatization

**REFERENCE**
Gilbert,J.D.; Olah,T.V.; Morris,M.J.; Bortnick,A.; Brunner,J. The use of stable isotope labeling and liquid chromatography-tandem mass spectrometry techniques to simultaneously determine the oral and ophthalmic bioavailability of timolol in dogs, *J.Chromatogr.Sci.*, **1998**, *36*, 163–168.

**SAMPLE**
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 296.1

**CHROMATOGRAM**
**Retention time:** 10.295

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate: benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane: acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25

mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylamino-sulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm Inertsil ODS-80A
**Mobile phase:** MeCN:water:trifluoroacetic acid 35:65:0.1
**Column temperature:** 40
**Flow rate:** 1
**Detector:** F ex 460 em 550

**CHROMATOGRAM**
**Retention time:** 28.8, 29.9 (enantiomers)
**Limit of detection:** 0.00595-0.00642 fmole

**OTHER SUBSTANCES**
**Also analyzed:** atenolol, carteolol

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

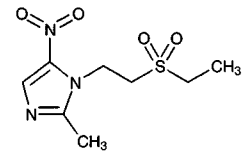
**SAMPLE**
**Matrix:** urine
**Sample preparation:** Mix 4 mL urine with 500μL 5 M NaOH, 4 mL diethyl ether and 1 g sodium sulfate. Shake the mixture mechanically for 15 min, centrifuge at 734 g for 5 min, separate, evaporate the diethyl layer to dryness at 60° under a gentle stream of nitrogen. Dissolve the residue in 2 mL mobile phase, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Guard column:** μBondapak C18
**Column:** 300 × 3.9 10 μm μBondapak C18
**Mobile phase:** MeCN:water 30:70 containing 5 mM pH 6.5 $KH_2PO_4/K_2HPO_4$
**Column temperature:** 30
**Flow rate:** 1.3
**Injection volume:** 20
**Detector:** E, PAR Model 400, glassy carbon electrode + 1 V, Ag/AgCl reference electrode

**CHROMATOGRAM**
**Retention time:** 6.47
**Limit of quantitation:** 10 ng/mL

**REFERENCE**
Maguregui,M.I.; Alonso,R.M.; Jiménez,R.M. A rapid quantitative analysis of the β-blocker timolol in human urine by HPLC-electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1643–1652.

# Tinidazole



**Molecular formula:** $C_8H_{13}N_3O_4S$
**Molecular weight:** 247.28
**CAS Registry No.:** 19387-91-8
**Merck Index:** 9588

## SAMPLE

**Matrix:** blood
**Sample preparation:** Add 50 μL 1M NaOH to 1 mL plasma, add 7 mL dichloromethane, mix on a rotary agitator for 20 min and centrifuge at 1636 g for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute the dry residue by vortex agitation with 200 μL mobile phase, inject a 50 μL aliquot.

## HPLC VARIABLES

**Column:** 250 × 4 10 μm Kromasil C8 (Bischoff Chromatography, Germany)
**Mobile phase:** MeCN:MeOH:68 mM pH 3 phosphate buffer 15:20:65
**Flow rate:** 0.7
**Injection volume:** 50
**Detector:** UV 360

## CHROMATOGRAM

**Retention time:** 6.10
**Internal standard:** tinidazole

## KEY WORDS

human; rat; plasma; tinidazole is internal standard

## REFERENCE

Enanga,B.; Labat,C.; Boudra,H.; Chauvière,G.; Keita,M.; Bouteille,B.; Dumas,M.; Houin,G. Simple high-performance liquid chromatographic method to analyse megazol in human and rat plasma, *J.Chromatogr.B*, **1997**, *696*, 261–266.

## SAMPLE

**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 317.6

## CHROMATOGRAM

**Retention time:** 10.563

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** gastric juice
**Sample preparation:** Mix 500 μL gastric juice with 50 μL 50% perchloric acid (w/v), vortex briefly, add 1.5 g solid anhydrous potassium carbonate to neutral pH. Add 300 μL MeCN, centrifuge at 11600 g for 6 min., remove a 180 μL aliquot of the supernatant, evaporate under a stream of nitrogen at 50°, reconstitute the residue with 500 μL mobile phase, inject a 100 μL aliquot.

**HPLC VARIABLES**
**Guard column:** 20 × 2 5 μm Hypersil ODS
**Column:** 150 × 4.6 5 μm Hypersil ODS
**Mobile phase:** MeCN:buffer 10:90 (Buffer was 50 mM $KH_2PO_4$ containing 0.1% triethylamine, adjusted to pH 7.0 with orthophosphoric acid.)
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 317

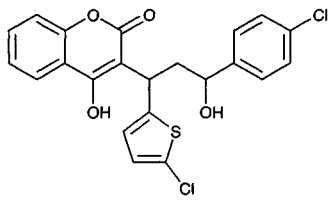
**CHROMATOGRAM**
**Retention time:** 13.9
**Internal standard:** tinidazole

**OTHER SUBSTANCES**
**Extracted:** metronidazole

**KEY WORDS**
tinidazole is IS

**REFERENCE**
Jessa,M.J.; Barrett,D.A.; Shaw,P.N.; Spiller,R.C. Rapid and selective high-performance liquid chromatographic method for the determination of metronidazole and its active metabolite in human plasma, saliva and gastric juice, *J.Chromatogr.B*, **1996**, *677*, 374–379.

# Tioclomarol




**Molecular formula:** $C_{22}H_{16}Cl2O_4S$
**Molecular weight:** 447.34
**CAS Registry No.:** 22619-35-8
**Merck Index:** 9594

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 4 μm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 312

## CHROMATOGRAM

**Retention time:** 9.41
**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

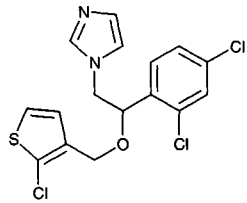
**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200.5

**CHROMATOGRAM**
**Retention time:** 22.525

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

# Tioconazole




**Molecular formula:** $C_{16}H_{13}Cl_3N_3OS$
**Molecular weight:** 387.72
**CAS Registry No.:** 65899-73-2
**Merck Index:** 9595

**SAMPLE**
**Matrix:** bulk
**Sample preparation:** Prepare a solution in mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm Hypersil phenyl
**Mobile phase:** MeCN:MeOH:buffer 17.4:40.6:42 (Buffer was 50 mM pH 4 triethylamine phosphate containing 25 mM 1-octanesulfonic acid.)
**Column temperature:** 40
**Flow rate:** 1.5
**Injection volume:** 20
**Detector:** UV 260

**CHROMATOGRAM**
**Retention time:** 7

**OTHER SUBSTANCES**
**Simultaneous:** impurities

**REFERENCE**
Wright,A.G.; Berridge,J.C.; Fell,A.F. Development and optimisation of a high-performance liquid chromatographic assay for tioconazole and its potential impurities. Part II. Selection of detection conditions for potential impurities, *Analyst*, **1989**, *114*, 53–56.

**SAMPLE**
**Matrix:** formulations

**Sample preparation:** Tablets. Powder tablets, weigh out amount equivalent to about 30 mg, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 100 μg/mL ketoconazole in MeOH, make up to 25 mL with MeOH, inject 20 μL aliquot. Cream. Condition a 500 mg Bond-Elut diol cartridge with 6 mL dichloromethane. Weigh out cream equivalent to about 5 mg of drug, add 30 mL dichloromethane, sonicate for 3 min, make up to 100 mL with dichloromethane, filter. Add a 2 mL aliquot to the cartridge, wash with 2 mL dichloromethane:methanol 4:1, wash with 2 mL dichloromethane, elute with 3 mL MeOH:buffer 85:15. Add eluate to 0.5 mL 100 μg/mL ketoconazole in MeOH, make up to 5 mL with MeOH, inject 20 μL aliquot. (Buffer was 50 mM triethylamine adjusted to pH 7.0 with phosphoric acid.)

### HPLC VARIABLES

**Column:** 250 × 4.6 5 μm Spherisorb CN
**Mobile phase:** THF:buffer 30:70 (Buffer was 50 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 230 [Enhanced sensitivity with photoreactor (Beam Boost model C6808 with 10 m × 0.3 mm reaction coil) followed by UV detection at 270 nm.]

### CHROMATOGRAM

**Retention time:** 12
**Internal standard:** ketoconazole (7)

### OTHER SUBSTANCES

**Simultaneous:** clotrimazole, ketoconazole, bifonazole, econazole, isoconazole, miconazole, fenticonazole

### KEY WORDS

tablets; creams; post-column photochemical derivatization

### REFERENCE

Di Pietra,A.M.; Cavrini,V.; Andrisano,V.; Gatti,R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 873–879.

### SAMPLE

**Matrix:** solutions
**Sample preparation:** Inject a 20 μL aliquot of a 100 μM solution.

### HPLC VARIABLES

**Column:** 250 × 4.6 Cyclobond 1 (β-cyclodextrin) (Astec)
**Mobile phase:** MeCN:buffer 10:90 (Buffer was 3.5% triethylamine adjusted to pH 4.0 with glacial acetic acid.)
**Column temperature:** 25
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 240

### CHROMATOGRAM

**Retention time:** 10.40 (-), 11.17 (+)

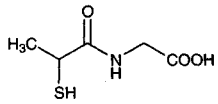
### KEY WORDS

chiral; comparison with capillary electrophoresis

### REFERENCE

Ferguson,P.D.; Goodall,D.M.; Loran,J.S. Systematic approach to the treatment of enantiomeric separations in capillary electrophoresis and liquid chromatography. III. A binding constant-retention factor relationship and effects of acetonitrile on the chiral separation of tioconazole, *J.Chromatogr.A*, **1996**, *745*, 25–35.

# Tiopronin



**Molecular formula:** $C_5H_9NO_3S$
**Molecular weight:** 163.20
**CAS Registry No.:** 1953-02-2
**Merck Index:** 9597

## SAMPLE

**Matrix:** blood
**Sample preparation:** 1 mL Plasma + 200 μL 200 mM pH 8.0 phosphate buffer + 200 μL chloroform:tributylphosphine 90:10, vortex for 15 s, heat at 50° for 30 min, cool in an ice bath, add 2 mL EtOH, vortex for 15 s, centrifuge at 5° at 1800 g for 10 min, inject a 50 μL aliquot of the supernatant within 15 min.

## HPLC VARIABLES

**Guard column:** 4 × 4 5 μm LiChrospher 100 RP-18e
**Column:** 125 × 4 5 μm LiChrospher 100 RP-18e
**Mobile phase:** MeCN:10 mM pH 7.0 phosphate buffer 25:75 containing 5 mM cetrimonium bromide
**Column temperature:** 35
**Flow rate:** 1
**Injection volume:** 50
**Detector:** F ex 260 em 370 (long-pass filter) following post-column reaction. The column effluent mixed with MeCN:water 30:70 containing 2% triethylamine and 1% Brij-35 pumped at 0.35 mL/min and 50 μM pyrenemaleimide in MeCN pumped at 0.35 mL/min and flowed through a 3 m × 0.5 mm i.d. PTFE knitted open tubular reactor to the detector.

## CHROMATOGRAM

**Retention time:** 6.5
**Limit of detection:** 50 ng/mL

## OTHER SUBSTANCES

**Extracted:** metabolites

## KEY WORDS

plasma; pharmacokinetics; post-column reaction

## REFERENCE

Leroy,P.; Nicolas,A.; Gavriloff,C.; Matt,M.; Netter,P.; Bannwarth,B.; Hercelin,B.; Mazza,M. Determination of 2-mercaptopropionylglycine and its metabolite, 2-mercaptopropionic acid, in plasma by ion-pair reversed-phase high-performance liquid chromatography with post-column derivatization, *J.Chromatogr.*, **1991**, *564*, 258–265.

## SAMPLE

**Matrix:** solutions
**Sample preparation:** Mix 10 μL 12 mM reagent in MeCN with 20 μL of a 30 μM solution of tiopronin in 2 mM $Na_2EDTA$ containing pyridine (final concentration 1%), heat at 50° for 60 min, inject a 10 μL aliquot. (Reagent was 4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole [R-(-)-DBD-PyNCS], available from Tokyo Kasei (TCI America, Portland, OR). Synthesis is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a

solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, was h, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 60F254 TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). DBD-F can also be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65?, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-Aminopyrrolidine is also available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

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**HPLC VARIABLES**

**Column:** 150 × 4.6 5 μm ULTRON VX-ODS (Shinwa Chemicals, Japan)

**Mobile phase:** MeCN:water containing 0.1% TFA 30:70 (A) or Gradient. C was MeCN containing 1% (v/v) TFA. D was water containing 1% TFA. C:D from 15:85 to 30:70 over 20min, to 40:60 over 30 min, maintain at 40:60 for 15 min (B)

**Flow rate:** 1

**Injection volume:** 10

**Detector:** F ex 455 em 568 (A), MS, Finnigan-MAT LCQ, ESI capillary temperature 275°, capillary voltage 3V, source voltage 4.8 kV, source current 100 μA, collision gas helium, positive ion mode, m/z 200-900 (B)

**CHROMATOGRAM**
**Retention time:** ca. 40 (R), 41 (S) (B)

**OTHER SUBSTANCES**
**Simultaneous:** hydrolysis product, reagent, D-cysteine, L- cysteine, (+)-2-mercaptopropionic acid, (-)-2-mercaptopropionic acid, N-(2-mercapto-2-methylpropionyl)-D-cysteine, N-(2-mercapto-2-methylpropionyl)-L-cysteine (B)
**Also analyzed:** N-acetyl-L-cysteine, N-acetyl-D/L-penicillamine, captopril, cysteamine, glutathione, D/L-homocysteine, 2-mercaptopropionic acid ethyl ester, D/L-penicillamine

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Jin,D.; Toyo'oka,T. Indirect resolution of thiol enantiomers by high-performance liquid chromatography with a fluorescent chiral tagging reagent, *Analyst*, **1998**, *123*, 1271–1277.

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**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Add 1.05-3 equivalents 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate to 10 mL of a 100 μM solution of the thiol in MeCN:water 50:50 containing 1-3 equivalents triethylamine, vortex briefly, let stand at room temperature for 30 min, dilute with mobile phase, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm TSKgel ODS-80TM (Tosoh)
**Mobile phase:** MeCN:10 mM pH 2.8 potassium phosphate buffer 50:50
**Column temperature:** 40
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 250

**CHROMATOGRAM**
**Retention time:** 2.12 (S), 2.63 (R)

**OTHER SUBSTANCES**
**Simultaneous:** alanine, mercaptopropionic acid

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Ito,S.; Ota,A.; Yamamoto,K.; Kawashima,Y. Resolution of the enantiomers of thiol compounds by reversed-phase liquid chromatography using chiral derivatization with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, *J.Chromatogr.*, **1992**, *626*, 187–196.

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**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Mix a 100 μL aliquot of a 0.1-1 mM solution in 10 mM HCl with 500 μL 100 mM pH 9.5 borate buffer, 100 μL 2.5 mM L-phenylalanine in 10 mM HCl, and 200 μL 5 mM o-phthalaldehyde in EtOH, let stand for 10 min, inject an aliquot.

**HPLC VARIABLES**
**Column:** 125 × 4 5 μm LiChrospher 100 RP-18 end-capped
**Mobile phase:** MeOH:50 mM pH 6.5 phosphate buffer 50:50
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 335

**CHROMATOGRAM**
**Retention time:** k' 1.9 ($\alpha$ = 2.01, $R_s$ = 5.18)

**KEY WORDS**
derivatization; chiral; comparison with capillary electrophoresis; comparison with other derivatizing reagents

**REFERENCE**
Leroy,P.; Bellucci,L.; Nicolas,A. Chiral derivatization for separation of racemic amino and thiol drugs by liquid chromatography and capillary electrophoresis, *Chirality*, **1995**, 7, 235–242.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Mix 20 μL of a 30 μM solution in 2 mM disodium EDTA containing 1.5% pyridine with 10 μL 12 mM R-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in MeCN, let stand for 40 min, inject a 10 μL aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole, (R)-(-)-NBD-PyNCS, is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL water, extract 4 times with 80 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as dark red crystals (mp 178-181°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as red crystals (mp 165-170°) (Analyst 1995, 120, 385).)

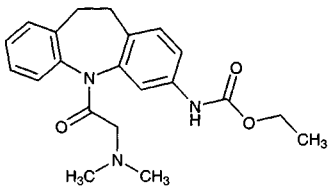
**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm Ultron VX-ODS (Shinwa, Kyoto)
**Mobile phase:** MeCN:water:trifluoroacetic acid 30:70:0.1
**Column temperature:** 40
**Flow rate:** 1
**Injection volume:** 10
**Detector:** F ex 455 em 568

**CHROMATOGRAM**
**Retention time:** 21.5 (R), 23 (S)
**Limit of detection:** 0.5 pmole

**KEY WORDS**
derivatization; chiral

**REFERENCE**
Jin,D.; Takehana,K.; Toyo'oka,T. Chiral separation of racemic thiols based on diastereomer formation with a fluorescent chiral tagging reagent by reversed-phase liquid chromatography, *Anal.Sci.*, **1997**, *13*, 113–115.

# Tiracizine



**Molecular formula:** $C_{21}H_{25}N_3O_3$
**Molecular weight:** 367.45
**CAS Registry No.:** 83275-56-3

## SAMPLE

**Matrix:** blood
**Sample preparation:** Add 1.5 mL MeCN to 500 μL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 μL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

## HPLC VARIABLES

**Column:** A 25 × 4 25 μm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 μm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
**Mobile phase:** MeCN:50 mM pH 4 $K_3PO_4$ buffer 27:73
**Column temperature:** 40
**Flow rate:** 1
**Injection volume:** 200
**Detector:** UV 242, UV 230

## CHROMATOGRAM

**Retention time:** 8.1

## OTHER SUBSTANCES

**Extracted:** celiprolol, metoprolol, talinolol, oxprenolol, metabolites

## KEY WORDS

serum; column-switching

## REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

## SAMPLE

**Matrix:** blood, urine
**Sample preparation:** Serum. 500 μL Serum + 500 ng talinolol + 1.5 mL MeCN, mix, centrifuge at 9000 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 250 μL water, inject a 200 μL aliquot on to column A and elute to waste with mobile phase A, after 4 min backflush the contents of column A on to column B with mobile phase B, elute with column B, monitor the effluent from column B. Urine. Inject a 50 μL aliquot of urine on to column A and elute to waste with mobile phase A, after 4 min backflush the contents of column A on to column B with mobile phase B, elute with column B, monitor the effluent from column B.

## HPLC VARIABLES

**Column:** A 30-40 μm Perisorb A (Merck); B 125 × 4 LiChroCART RP18 (Merck)
**Mobile phase:** A water; B MeCN:50 mM pH 4 phosphate buffer 27:73
**Flow rate:** A 1.4; B 1
**Injection volume:** 200
**Detector:** UV 230

## CHROMATOGRAM

**Internal standard:** talinolol
**Limit of quantitation:** 200 ng/mL (urine), 10 ng/mL (serum)

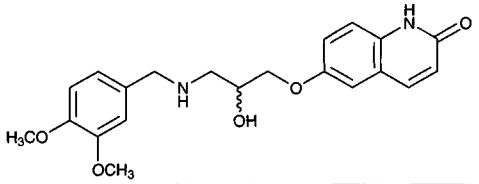
**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
serum; pharmacokinetics; column-switching

**REFERENCE**
Berndt,A.; Oertel,R.; Terhaag,B.; Richter,K.; Gramatté,T. Pharmacokinetics of the antiarrhythmic agent tiracizine: Steady state kinetics in comparison with single-dose kinetics, *Biopharm.Drug Dispos.*, **1995**, *16*, 427–441.

# Toborinone

**Molecular formula:** $C_{21}H_{24}N_2O_5$
**Molecular weight:** 384.43
**CAS Registry No.:** 143343-83-3




**SAMPLE**
**Matrix:** blood, tissue, urine
**Sample preparation:** Plasma, tissue. Mix plasma or homogenized tissue samples with MeOH: MeCN 50:50, sonicate, centrifuge at 1800 g for 5 min. Remove the supernatant, re-extract the residue 4 times. Combine the supernatants and lyophilize. Reconstitute the residue in mobile phase (A:B 50:50), inject an aliquot. Feces. Mix fecal homogenate with MeOH/water, extract 6 times, combine the supernatants and lyophilize. Reconstitute the residue in mobile phase(A:B 50:50), inject an aliquot. Urine. Inject urine directly or lyophilize and reconstitute in mobile phase (A:B 50:50), inject an aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 Develosil ODS-5(Nomura, Japan)
**Mobile phase:** Gradient. A:B from 100:0 to 90:10 over 20 min, maintain at 90:10 for 10 min, to 70:30 over 10 min, to 50:50 over 5 min and back to 100:0 over 0.1 min. A was MeCN:MeOH: water:acetic acid 5:5:90:0.5. B was MeCN:MeOH:water:acetic acid 25:25:50:0.5.
**Column temperature:** 30
**Flow rate:** 1
**Detector:** F ex 355 em 405

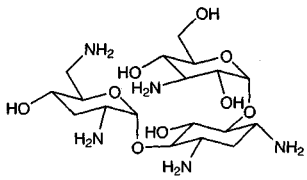
**CHROMATOGRAM**
**Retention time:** 40

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
rat; dog; plasma; feces; liver; details of analytical and preparative HPLC

**REFERENCE**
Kitani,M.; Miyamoto,G.; Nagasawa,M.; Yamada,T.; Matsubara,J.; Uchida,M.; Odomi,M. Biotransformation of the novel inotropic agent toborinone (OPC-18790) in rats and dogs. Evidence for the formation of novel glutathione and two cysteine conjugates, *Drug Metab.Dispos.*, **1997**, *25*, 663–674.

# Tobramycin



**Molecular formula:** $C_{18}H_{37}N_5O_9$
**Molecular weight:** 467.52
**CAS Registry No.:** 32986-56-4, 79645-27-5 (sulfate)
**Merck Index:** 9628

## SAMPLE

**Matrix:** blood
**Sample preparation:** Condition a Bond-Elut C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of water. 50 μL Serum + 25 μL 2 M pH 10.3 Tris buffer + 100 μL 10 μg/mL sisomicin in MeCN, vortex, centrifuge at 15000 g for 1 min. Remove the supernatant and add it to 30 μL 250 mg/mL 2,4,6-trinitrobenzenesulfonic acid in MeCN, vortex, heat at 70° for 30 min. Add 700 μL wash solution then 200 μL sample to the SPE cartridge, wash with 3 column volumes of wash solution, elute with 300 μL MeCN, inject a 50 μL aliquot of the eluate. (Prepare wash solution by adding 10 mL 1 M $K_2HPO_4$ to 90 mL water, add 100 mL MeOH, adjust pH to 8.5 with phosphoric acid.)

## HPLC VARIABLES

**Column:** 250 × 4.6 Ultrasphere octyl
**Mobile phase:** MeCN:buffer 70:30 (Buffer was 6.8 g/L $KH_2PO_4$ adjusted to pH 3.5 with phosphoric acid.)
**Column temperature:** 50
**Flow rate:** 3
**Injection volume:** 50
**Detector:** UV 340

## CHROMATOGRAM

**Retention time:** 3
**Internal standard:** sisomicin (4)
**Limit of detection:** 1200 ng/mL

## OTHER SUBSTANCES

**Simultaneous:** gentamicin
**Noninterfering:** acetaminophen, acetazolamide, N-acetylprocainamide, amikacin, amobarbital, ampicillin, amitriptyline, caffeine, cefamandole, cefoxime, cefoxitin, cephalothin, clindamycin, chloramphenicol, chlordiazepoxide, diazepam, erythromycin, ethosuximide, glutethimide, imipramine, kanamycin, methaqualone, moxalactam, nafcillin, nitrofurantoin, penicillin G, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, secobarbital, tetracycline, theophylline, vancomycin

## KEY WORDS

serum; derivatization; SPE

## REFERENCE

Kabra,P.M.; Bhatnagar,P.K.; Nelson,M.A.; Wall,J.H.; Marton,L.J. Liquid-chromatographic determination of tobramycin in serum with spectrophotometric detection, *Clin.Chem.*, **1983**, *29*, 672–674.

## SAMPLE

**Matrix:** blood
**Sample preparation:** Condition a Bond Elut C18 SPE cartridge with 1 mL MeCN and 1 mL MeCN:20 mM pH 8 phosphate buffer 10:90. 100 μL Serum + 400 μL water + 500 μL 10% sulfosalicylic acid, vortex, centrifuge. Remove 60 μL supernatant and add it to 240 μL MeCN and 300 μL o-phthalaldehyde reagent, add to SPE cartridge, rinse in with 300 μL rinse + 100 μL MeCN:20 mM pH 8 phosphate buffer 10:90, wash with 500 μL MeCN:20 mM pH 8 phosphate buffer 10:90, elute with 440 μL MeCN, add 40 μL water to eluate, vortex, inject a 25 μL aliquot. (o-Phthalaldehyde reagent was 200 mg o-phthalaldehyde in 2 mL MeOH + 400 μL 2-mercaptoethanol. Mix with 1 g boric acid in 38 mL water adjusted to pH 10.4 with 450 g/L KOH, store under nitrogen at 4°.)

**HPLC VARIABLES**
**Column:** 150 × 4 MicroPak SP C8 (Varian)
**Mobile phase:** MeCN:20 mM pH 6.5 phosphate buffer 48:52
**Flow rate:** 2
**Injection volume:** 25
**Detector:** F ex 340 em 450

**CHROMATOGRAM**
**Retention time:** 8.4
**Limit of detection:** 10 pmol

**KEY WORDS**
serum; SPE; derivatization

**REFERENCE**
Lai,F.; Sheehan,T. Enhancement of detection sensitivity and cleanup selectivity for tobramycin through pre-column derivatization, *J.Chromatogr.*, **1992**, *609*, 173–179.

**SAMPLE**
**Matrix:** blood, dialysate, urine
**Sample preparation:** Plasma. Condition a 3 mL Baker cyanopropylsilane CN SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer. 1 mL Plasma + 100 μL 100 μg/mL dibekacin in water, vortex for 15 s, add 1 mL buffer, vortex for 15 s, centrifuge at 3100 g at 4° for 7 min, add to SPE cartridge, wash with 500 μL water, wash with 250 μL mobile phase, elute to dryness. Elute with 250 μL mobile phase, inject an aliquot of the eluate. Urine, dialysate. Dilute 1:100 with water, add 100 μL 100 μg/mL dibekacin per 1 mL of sample, mix well, inject a 100 μL aliquot. (Buffer was 0.94 g sodium hexanesulfonate in 300 mL water, add 500 μL glacial acetic acid, dilute to 500 mL with water.)

**HPLC VARIABLES**
**Guard column:** 10 × 4.6 5 μm Hypersil C18
**Column:** 150 × 4.6 5 μm Hypersil C18
**Mobile phase:** MeOH:buffer 10:90 (Buffer was 3.76 g sodium hexanesulfonate + 28.4 g sodium sulfate in 2 L water, acidify to pH 3.4 with 2 mL glacial acetic acid.)
**Column temperature:** 25
**Flow rate:** 1.1
**Injection volume:** 100
**Detector:** F ex 338 em 418 (bandpass filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.05 mm i.d. knitted PTFE reaction coil at 25° to the detector (Derivatizing reagent was 0.4 g o-phthalaldehyde in 3 mL MeOH added to 390 mL buffer, add 2 mL β-mercaptoethanol, make up to 500 mL with water, store at 4°. Buffer was 1 M pH 10.4 borate from equal volumes of 1 M KOH and boric acid.)

**CHROMATOGRAM**
**Retention time:** 13
**Internal standard:** dibekacin (17)

**OTHER SUBSTANCES**
**Simultaneous:** isepamicin, kanamycin, gentamicin, netilmicin

**KEY WORDS**
post-column reaction; SPE; plasma

**REFERENCE**
Maloney,J.A.; Awni,W.M. High-performance liquid chromatographic determination of isepamicin in plasma, urine and dialysate, *J.Chromatogr.*, **1990**, *526*, 487–496.

**SAMPLE**
**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 200.5

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**CHROMATOGRAM**
**Retention time:** 13.393

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**KEY WORDS**
whole blood

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**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

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**SAMPLE**
**Matrix:** bulk, formulations
**Sample preparation:** Dilute 500 μL injection to 25 mL with Sorensen's phosphate buffer, dilute a 1 mL aliquot to 100 mL with Sorensen's phosphate buffer. Dissolve tobramycin-containing polymethylmethacrylate beads in 2 mL chloroform, extract 3 times with 5 mL aliquots of 50 mM $KH_2PO_4$, combine and dilute the extracts. Dilute bulk samples with Sorensen's phosphate buffer. 50 μL Solution + 25 μL 242 mg/mL pH 10.4 Tris buffer + 100 μL 6 μg/mL kanamycin in MeCN:water 50:50 + 30 μL 250 mg/mL 2,4,6-trinitrobenzenesulfonic acid in MeCN:water 80:20, vortex for 10 s, heat at 70° for 15 min, add 2 mL chloroform, shake horizontally at 180 cycles/min for 5 min, centrifuge at 750 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN, vortex, inject a 20 μL aliquot. (Sorensen's phosphate buffer was 197 mL 9.08 g/L $KH_2PO_4$ and 1803 mL 11.88 g/L $Na_2HPO_4.2H_2O$, pH 7.4.)

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**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm Ultrasphere octyl
**Mobile phase:** MeCN:50 mM $KH_2PO_4$ 62:38, pH adjusted to 3.5 with phosphoric acid
**Flow rate:** 2.5
**Injection volume:** 20
**Detector:** UV 340

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**CHROMATOGRAM**
**Retention time:** 7.7
**Internal standard:** kanamycin (6.4)
**Limit of detection:** 160 ng/mL
**Limit of quantitation:** 780 ng/mL

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**KEY WORDS**
injections; beads; derivatization

**REFERENCE**
Dash,A.K.; Suryanarayanan,R. A liquid-chromatographic method for the determination of tobramycin, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 237–245.

**SAMPLE**
**Matrix:** fermentation solutions
**Sample preparation:** 5 mL Fermentation broth + 5 mL saturated aqueous solution of Tris + 20 mL MeCN, centrifuge at 3000 rpm for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 3 mL 150 mM 2,4-dinitrofluorobenzene in MeOH, heat at 100° under a reflux condenser for 45 min, make up to 4 mL with mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Column:** 200 × 4.6 10 μm LiChrosorb RP-8
**Mobile phase:** MeCN:water:acetic acid 55:45:0.15
**Flow rate:** 1.2
**Injection volume:** 20
**Detector:** UV 350

**CHROMATOGRAM**
**Retention time:** 14.86

**OTHER SUBSTANCES**
**Extracted:** apramycin, kanamycin B

**KEY WORDS**
derivatization

**REFERENCE**
Harangi,J.; Deák,M.; Nánási,P.; Bacsa,G. Determination of the major factors of fermentation of the nebramycin complex by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 83–93.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute 3 mg/mL ophthalmic suspension in water with 10 mM sulfuric acid to a tobramycin concentration of 240 μg/mL. Mix 4 mL diluted suspension with 10 mL 10 mg/mL 2,4-dinitrofluorobenzene in EtOH and 10 mL 15 mg/mL tris(hydroxymethyl)aminomethane in water:dimethylsulfoxide 20:80. Heat at 70 ± 2°. for 20 min, allow to cool slightly for 2 min and add 24 mL MeCN. Allow to cool to room temperature, make up to 50 mL with MeCN, inject a 30 μL aliquot.

**HPLC VARIABLES**
**Column:** 150 × 3.9 Nova-Pak C18
**Mobile phase:** MeCN:buffer 55:45 (Prepare mobile phase as follows. Dissolve 2.0 g tris (hydroxymethyl)aminomethane in 960 mL water, add 20 mL 0.5 M sulfuric acid and 1200 mL MeCN.)
**Flow rate:** 1.5
**Injection volume:** 30
**Detector:** UV 365

**CHROMATOGRAM**
**Retention time:** 9.0
**Limit of quantitation:** 0.1%

**OTHER SUBSTANCES**
**Simultaneous:** degradation products, kanamycin, neamine, nebramine

**KEY WORDS**
derivatization; ophthalmic suspension; stability-indicating

### REFERENCE

Russ,H.; McCleary,D.; Katimy,R.; Montana,J.L.; Miller,R.B.; Krishnamoorthy,R.; Davis,C.W. Development and validation of a stability-indicating HPLC method for the determination of tobramycin and its related substances in an ophthalmic suspension, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2165–2181.

### SAMPLE

**Matrix:** formulations
**Sample preparation:** Dilute 150 μL sample to 5 mL, inject an aliquot.

### HPLC VARIABLES

**Guard column:** C18 precolumn filter
**Column:** 150 × 3.9 4 μm Nova Pak C18
**Mobile phase:** MeCN:200 mM $KH_2PO_4$ 30:70 adjusted to pH 6.5
**Flow rate:** 1
**Detector:** UV 210

### CHROMATOGRAM

**Retention time:** 6.78

### OTHER SUBSTANCES

**Simultaneous:** degradation products

### KEY WORDS

stability-indicating; ophthalmic solutions

### REFERENCE

McBride,H.A.; Martinez,D.R.; Trang,J.M.; Lander,R.D.; Helms,H.A. Stability of gentamicin sulfate and tobramycin sulfate in extemporaneously prepared ophthalmic solutions at 8 degrees C, *Am.J.Hosp.Pharm.*, **1991**, *48*, 507–509.

### SAMPLE

**Matrix:** formulations, tissue
**Sample preparation:** Homogenize (Polytron) kidney or lung tissue with 2.5 volumes cold sterile PBS for 30 s. 100 μL Tissue homogenate or liposome encapsulations + 1 mL MeOH, vortex for 1 min, heat at 65° for 30 min, add 900 μL PBS, vortex for 1 min, centrifuge at 4° at 5000 g for 20 min. Remove a 170 μL aliquot of the supernatant and add it to 90 μL 180 mg/mL 1-fluoro-2,4-dinitrofluorobenzene in MeOH, add 60 μL 100 mM pH 9.3 borate buffer, add 670 μL MeOH, vortex, heat at 85° for 30 min, cool to room temperature, inject an aliquot.

### HPLC VARIABLES

**Column:** 250 × 4.6 4 μm Zorbax SB-C18
**Mobile phase:** MeCN:MeOH:10 mM potassium phosphate buffer 65:10:25, pH 3.5
**Flow rate:** 1.3
**Injection volume:** 10
**Detector:** UV 350

### CHROMATOGRAM

**Limit of detection:** 200 ng/mL (PBS), 300 ng/mg (lung), 500 ng/mg (kidney)

### KEY WORDS

rat; derivatization; lung; kidney; liposome encapsulations

### REFERENCE

Beaulac,C.; Clement-Major,S.; Hawari,J.; Lagace,J. Eradication of mucoid Pseudomonas aeruginosa with fluid liposome-encapsulated tobramycin in an animal model of chronic pulmonary infection, *Antimicrob.Agents Chemother.*, **1996**, *40*, 665–669.

### SAMPLE

**Matrix:** reaction mixtures

**Sample preparation:** 50 μL Buffered reaction mixture + 50 μL isopropanol + 50 μL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 μL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

**HPLC VARIABLES**
**Column:** 100 × 5 Hypersil ODS
**Mobile phase:** A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 55:45.
**Flow rate:** 2
**Injection volume:** 50
**Detector:** UV 330

**CHROMATOGRAM**
**Retention time:** 18

**KEY WORDS**
derivatizaton

**REFERENCE**
Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject a 25 μL aliquot.

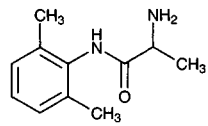
**HPLC VARIABLES**
**Guard column:** RCSS Guard-Pak (Waters)
**Column:** 100 × 8 C18 Radial Pak (Waters)
**Mobile phase:** MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
**Flow rate:** 3
**Injection volume:** 25
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 5.5

**OTHER SUBSTANCES**
**Simultaneous:** acetaminophen, N-acetylprocainamide, cefaclor, cefamandole, cefazolin, cefotaxime, cefoxitin, cephalexin, cephalothin, cephapirin, chloramphenicol, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, vancomycin

**REFERENCE**
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

# Tocainide




**Molecular formula:** $C_{11}H_{16}N_2O$
**Molecular weight:** 192.26
**CAS Registry No.:** 41708-72-9
**Merck Index:** 9629
**Lednicer No.:** 3 55

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 1.0 mL Serum + 500 μL saturated borate buffer + 3.0 mL dichloromethane, vortex, centrifuge. Evaporate the organic layer to dryness under a stream of nitrogen at 40°. Reconstitute with mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Guard column:** silica (Whatman)
**Column:** 250 × 4.6 Supelcosil LC-8-DB
**Mobile phase:** MeCN:20 mM phosphoric acid containing 200 μL/L triethylamine 10:90
**Flow rate:** 1.7
**Detector:** UV 263

**CHROMATOGRAM**
**Retention time:** 9.7
**Internal standard:** tocainide

**OTHER SUBSTANCES**
**Extracted:** lidocaine
**Noninterfering:** acetaminophen, N-acetylprocainamide, amitriptyline, bupivicaine, caffeine, carbamazepine, chloramphenicol, cyclosporin A, desipramine, diazepam, disopyramide, doxepin, ethosuximide, flecainide, fluoxetine, ibuprofen, imipramine, naproxen, norchlordiazepoxide, nordiazepam, nordoxepine, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, valproic acid

**KEY WORDS**
serum; tocainide is IS

**REFERENCE**
O'Neal,C.L.; Poklis,A. Sensitive HPLC for simultaneous quantification of lidocaine and its metabolites monoethylglycinexylidide and glycinexylidide in serum, *Clin.Chem.*, **1996**, *42*, 330–331.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** 0.05-2 mL Plasma or whole blood + 50 μL 13.5 μg/mL IS in 50 mM HCl + 200 μL 2 M NaOH + 3 mL ethyl acetate, mix with gentle tilting for 5 min, centrifuge at 2500 rpm for 10 min. Remove 2 mL of the organic layer and add it to 50 μL 50 mM sulfuric acid, vortex for 1 min, centrifuge for 5 min, discard the organic phase. Wash the aqueous phase with 1 mL hexane, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add 50 μL 1 M sodium bicarbonate and 200 μL reagent, heat at 40° for 30 min, add 100 μL 500 mM NaOH, evaporate under reduced pressure at 40° for 3 min, add 1 mL buffer and 200 μL carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), mix for 2 min, centrifuge for 5 min, inject a 2-5 μL aliquot of the organic layer. (Reagent was 1 mg/mL dansyl chloride in acetone, store in the dark, prepare fresh each week. Buffer was a half-saturated solution of disodium citrate in water, adjusted to pH 6.0 with 85% phosphoric acid.)

**HPLC VARIABLES**
**Column:** 300 × 4 μBondapak NH2
**Mobile phase:** Hexane:dichloromethane:MeOH 50:50:1
**Flow rate:** 5
**Injection volume:** 2-5
**Detector:** F ex 360 (Corning 7-51 filter) em 490 (Wratten 8 filter)

**CHROMATOGRAM**
**Retention time:** 2.4
**Internal standard:** 2-amino-N-(2,6-dimethylphenyl)butanamide hydrochloride (Astra Pharmaceuticals, Worcester, MA) (1.9)
**Limit of quantitation:** 100 ng/mL

**KEY WORDS**
derivatization; plasma; whole blood; pharmacokinetics

**REFERENCE**
Meffin,P.J.; Harapat,S.R.; Harrison,D.C. High-pressure liquid chromatographic analysis of drugs in biological fluids II: Determination of an antiarrhythmic drug, tocainide, as its dansyl derivative using a fluorescence detector, *J.Pharm.Sci.*, **1977**, *66*, 583–586.

### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 1 mL pH 11 sodium borate buffer + 2.5 mL diisopropyl ether:EtOH 100:1.5 (Caution! Diisopropyl ether readily forms explosive peroxides!), shake for 30 min, centrifuge, repeat the extraction. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 100 μL toluene, evaporate to dryness, add 100 μL 400 μg/mL S-naproxen chloride in anhydrous dichloromethane, heat at 50° for 1 h, evaporate to dryness, reconstitute with mobile phase, inject an aliquot (cf Arch. Pharm. (Weinheim) 1990, 323, 465). (Synthesis of S-naproxen chloride is as follows. Protect all compounds from light. Dissolve 500 mg naproxen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 1 h, evaporate to dryness under reduced pressure, dry over KOH under vacuum overnight to obtain S-naproxen chloride (mp 96°).)

### HPLC VARIABLES

**Column:** 250 × 4.6 5 μm Zorbax Sil
**Mobile phase:** Cyclohexane:dichloromethane:THF 50:20:20
**Detector:** F ex 313 em 365

### CHROMATOGRAM

**Retention time:** k' 4.0 (R), k' 5.2 (S)

### KEY WORDS

derivatization; protect from light; chiral; plasma; normal phase

### REFERENCE

Spahn,H. S-(+)-Naproxen chloride as acylating agent for separating the enantiomers of chiral amines and alcohols, *Arch.Pharm.(Weinheim)*, **1988**, *321*, 847–850.

### SAMPLE

**Matrix:** blood

**Sample preparation:** 500 μL Plasma + 25 μL 10 μg/mL acebutolol in water + 200 μL 1 M NaOH + 5 mL chloroform, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL 0.05% S-(+)-1-(1-naphthyl)ethylisocyanate in chloroform, vortex for 30 s, evaporate to dryness under reduced pressure, reconstitute with 200 μL chloroform, inject a 50-175 μL aliquot.

### HPLC VARIABLES

**Column:** 250 × 4.6 Partisil 5 silica
**Mobile phase:** Hexane:chloroform:MeOH 60:38:2
**Flow rate:** 2
**Injection volume:** 50-175
**Detector:** F ex 220 em 345

### CHROMATOGRAM

**Retention time:** 3.5 (S), 4.5 (R) (tentative assignment)
**Internal standard:** (±)-acebutolol (14.9 (R), 16.5 (S))
**Limit of detection:** 25 ng/mL
**Limit of quantitation:** 250 ng/mL

### KEY WORDS

plasma; derivatization; chiral; pharmacokinetics

### REFERENCE

Carr,R.A.; Foster,R.T.; Freitag,D.; Pasutto,F.M. Stereospecific high-performance liquid chromatographic determination of tocainide, *J.Chromatogr.*, **1991**, *566*, 155–162.

### SAMPLE

**Matrix:** blood

**Sample preparation:** 100 μL Serum or plasma + 100 μL 500 mM sodium carbonate + 100 μL 15 μg/mL N-propionylprocainamide in water, vortex for 5 s, add 0.5 (procainamide) or 1 (tocainide) mL dichloromethane, vortex for 30 (procainamide) or 60 (tocainide) s, centrifuge at

9500 g for 1 min. Remove the lower organic layer and add it to 200 μL 10 mM HCl, vortex for 15 s, centrifuge, inject a 20 μL aliquot of the aqueous layer.

**HPLC VARIABLES**
**Column:** 100 × 5 NovaPak cyano HP radial compression
**Mobile phase:** MeCN:buffer 10:90, final pH adjusted to 6.0 (Buffer was 5 mM acetate buffer containing 0.05% triethylamine.)
**Flow rate:** 1.5
**Injection volume:** 20
**Detector:** UV 280

**CHROMATOGRAM**
**Retention time:** 3.1
**Internal standard:** N-propionylprocainamide (6.1)
**Limit of detection:** 2 μg/mL

**OTHER SUBSTANCES**
**Extracted:** N-acetylprocainamide, procainamide
**Simultaneous:** disopyramide, lidocaine, mexiletine, quinidine
**Noninterfering:** carbamazepine, desmethyldoxepin, digoxin, doxepin, ethosuximide, lithium, phenobarbital, phenytoin, primidone, propranolol, theophylline, valproic acid

**KEY WORDS**
serum; plasma

**REFERENCE**
vasBinder,E.; Annesley,T. Liquid chromatographic analysis of mexiletine in serum, with alternate application to tocainide, procainamide, and N-acetylprocainamide, *Biomed.Chromatogr.*, **1991**, *5*, 19–22.

**SAMPLE**
**Matrix:** blood
**Sample preparation:** Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 20 mM pH 10 potassium phosphate buffer. 1 mL Plasma + 25 μL 1.2 mg/mL benzylamine hydrochloride in water + 100 mg solid sodium carbonate, vortex, add to the SPE cartridge, wash with 1 mL 20 mM pH 10 potassium phosphate buffer, elute with 500 μL MeOH. Add 20 μL 1 M (-)-menthyl chloroformate in MeCN to the eluate, shake briefly, let stand for 3 min, inject an aliquot.

**HPLC VARIABLES**
**Column:** 120 × 4.6 5 μm Spherisorb ODS-2
**Mobile phase:** MeOH:200 mM pH 5.0 potassium phosphate buffer:water 75:2.5:22.5
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 262

**CHROMATOGRAM**
**Retention time:** 4.70 (R), 5.07 (S)
**Internal standard:** benzylamine (6.22)
**Limit of quantitation:** 1 μg/mL

**KEY WORDS**
chiral; derivatization; SPE; plasma; rabbit; pharmacokinetics

**REFERENCE**
Christensen,E.B.; Hansen,S.H.; Rasmussen,S.N. Assay of tocainide enantiomers in plasma by solid-phase extraction and indirect chiral high-performance liquid chromatography after derivatization with (-)-menthyl chloroformate, *J.Chromatogr.B*, **1995**, *670*, 243–249.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
**Flow rate:** 2
**Injection volume:** 20
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 1.9

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

## SAMPLE

**Matrix:** solutions
**Sample preparation:** 50 μL 5 mg/mL Tocainide in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer was 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 16.3 mg N-acetyl-L-cysteine in 1 mL MeOH, protect from light, keep on ice.)

### HPLC VARIABLES
**Column:** 150 × 3.9 4 μm Nova-Pak C18
**Mobile phase:** MeOH:MeCN:buffer 40:2:60 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
**Flow rate:** 1
**Injection volume:** 5
**Detector:** F ex 338 em 425 or UV 254

### CHROMATOGRAM
**Retention time:** 20.32 (S-(+)), 22.47 (R-(-))

### KEY WORDS
derivatization; protect from light; chiral

### REFERENCE
Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

### SAMPLE
**Matrix:** solutions

### HPLC VARIABLES
**Column:** 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
**Flow rate:** 0.6
**Injection volume:** 25
**Detector:** UV 229

### CHROMATOGRAM
**Retention time:** 6.28 (A), 3.60 (B)

### OTHER SUBSTANCES
**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupro-

mazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

**KEY WORDS**
details of plasma extraction

**REFERENCE**
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Mix 20 μL of a 1 mM solution in MeOH or water with 50 μL pH 8 borate buffer and 50 μL 18 mM 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate in acetone, vortex, let stand at room temperature for 30 min, add 100 μL 10 mM trans-4-hydroxy-L-proline in water, mix, let stand for 2 min, add 2 mL dichloromethane, vortex for 30 s. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL mobile phase, inject an aliquot. Prepare 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate as follows. Stir 1.5 mmoles lithium aluminum hydride in THF, slowly add 2 mmoles (S)-naproxen in 20 mL anhydrous THF, reflux for 1 h, evaporate most of the solvent, cautiously add water with stirring, acidify with 6 N HCl, extract three times with diethyl ether. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane:MeOH 100:2 (flash chromatography), evaporate eluate to dryness, dry under vacuum over KOH to give 2-(6-methoxy-2-naphthyl)propanol as a white solid (mp 92-3°). Stir 0.5 mmoles 2-(6-methoxy-2-naphthyl)propanol and 0.5 mmoles triethylamine in 10 mL dry toluene at 0°, add 1 mL 20% phosgene in toluene (Caution! Phosgene is highly toxic, perform reaction in a chemical fume hood!) (Fluka), stir for 4 h, filter, evaporate to dryness under reduced pressure, dry under vacuum to give 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (mp 60°). Store under vacuum over phosphorus pentoxide at room temperature.)

**HPLC VARIABLES**
**Column:** 250 × 4 5 μm Zorbax-SIL
**Mobile phase:** n-Hexane:isopropanol 100:1.5
**Flow rate:** 1.5
**Injection volume:** 100
**Detector:** UV 230, F ex 270 em 365

**CHROMATOGRAM**
**Retention time:** k' 15.7 (S-(+)), k' 16.9 (R-(-))

**OTHER SUBSTANCES**
**Simultaneous:** flecainide, metoprolol, propafenone

**KEY WORDS**
derivatization; chiral; normal phase

**REFERENCE**
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene

or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

**HPLC VARIABLES**
**Column:** 250 × 4 5 μm Zorbax ODS
**Mobile phase:** MeOH:water 70:30
**Flow rate:** 0.8
**Injection volume:** 100
**Detector:** UV 230, F ex 270 em 350

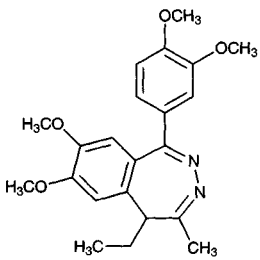
**CHROMATOGRAM**
**Retention time:** k' 10.1 (S-(+)), 11.1 (R-(-))

**KEY WORDS**
derivatization; chiral; F not much more sensitive than UV; α = 1.10

**REFERENCE**
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

# Tofisopam

**Molecular formula:** $C_{22}H_{26}N_2O_4$
**Molecular weight:** 382.46
**CAS Registry No.:** 22345-47-7




**SAMPLE**
**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

**HPLC VARIABLES**
**Column:** 300 × 3.9 4 μm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50

**Detector:** UV 238

## CHROMATOGRAM

**Retention time:** 4.04
**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, urine
**Sample preparation:** 4.0 mL Blood, plasma, or urine + 1.5 mL pH 8.0 dibasic ammonium phosphate + 4.5 mL dichloromethane, gently shake horizontally for 10 min, centrifuge at 3500 g for 10 min. Transfer the lower organic layer to 5 mL tube and evaporate under reduced pressure at 45° to 1.0 mL. Transfer into 1.5 mL Eppendorff-type plastic microtube and evaporate to dryness. Add 30 μL mobile phase, vortex for 10 s, centrifuge at 10 000 g for 5 min, inject a 0.6 μL aliquot of the supernatant. (Equilibrate the column at least 3 h before analyzing. At the end of each chromatographic session clean column with MeCN:water 50:50 at 0.05 mL/min for 3 h.)

## HPLC VARIABLES

**Guard column:** 1.0 × 0.8 5 μm C18 MGU-80 (LC Packing, Switzerland)
**Column:** 250 × 1.0 5 μm C18 Microbore (Alltech, USA)
**Mobile phase:** MeCN:2 mM pH 3.0 ammonium formate 75:25
**Flow rate:** 0.05

**Injection volume:** 0.6
**Detector:** MS, Perkin-Elmer Sciex API-100 double-quadrupole, OR + 50 V, Q0–10 V, IQ1 (lens)–12 V, ST (lens)–15 V, Q1–13 V, EM + 2200 V, TIC m/z 100-500 or 380-405, SIM m/z 383 pm 0.5

### CHROMATOGRAM
**Retention time:** 4.53
**Internal standard:** tofisopam

### OTHER SUBSTANCES
**Extracted:** colchicine

### KEY WORDS
plasma; tofisopam is IS; microbore; use PEEK tubing and injection loop

### REFERENCE
Tracqui,A.; Kintz,P.; Ludes,B.; Rouge,C.; Douibi,H.; Mangin,P. High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids, *J.Chromatogr.B*, **1996**, *675*, 235–242.

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### SAMPLE
**Matrix:** blood, urine
**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

### HPLC VARIABLES
**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 204

### CHROMATOGRAM
**Retention time:** 18.508

### KEY WORDS
whole blood

### REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.